

*****STN Columbus *****
FILE 'HOME' ENTERED AT 14:27:12 ON 04 MAR 2003
=> d his

(FILE 'HOME' ENTERED AT 14:27:12 ON 04 MAR 2003)

FILE 'REGISTRY' ENTERED AT 14:27:28 ON 04 MAR 2003

L1 1 SLDAO

FILE 'CA' ENTERED AT 14:27:38 ON 04 MAR 2003

L2 1579 SL1

L3 1 S HEPATITIS AND L2

L4 9599 S REFOLED? OR RENATUR?

L5 3 SL2 AND L4

L6 115 S PROTEIN AND L2

L7 8 S INCLUSION AND L2

FILE 'REGISTRY' ENTERED AT 14:30:58 ON 04 MAR 2003

=> d13 bib abs

L3 ANSWER 1 OF 1 CA COPYRIGHT 2003 ACS
AN 137:29823 CA

TI Purification of active NS2/3 protease of ***hepatitis*** C virus from
inclusion bodies

IN Thibeault, Diane; Lamarte, Daniel; Maurice, Roger; Pilote, Louise; Pause,
Amin

PA Boehringer Ingelheim (Canada) Ltd., Can.

SO PCT Int. Appl., 67 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN CNT 1

PATENT NO. KIND DATE APPLICATION NO. DATE

PI WO 2002048375 A2 20020620 WO 2001-CA1796 20011213

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL,
PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG,
US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH,
CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR,
BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

AU 2002024688 A5 20020624 AU 2002-24688 20011213
US 2002192640 A1 20021219 US 2001-17736 20011214

PRAI US 2000-256031P P 20001215
WO 2001-CA1796 W 20011213

AB A method for producing a refolded, inactive form of recombinantly produced
NS2/3 protease by purifying the protease from inclusion bodies in the
presence of a chaotropic agent and refolding the purified protease by

contacting it with a reducing agent and lauryldiethylamine oxide (LDAO) in
the presence of reduced concn. of chaotropic agent or polar additive. The
invention further comprises a method for activating this refolded inactive
NS2/3 protease by adding an activation detergent. This method produces
large ams. of the active NS2/3 protease to allow small moles and ligands
to be screened as potential inhibitors of NS2/3 protease, which may be
useful as therapeutic agents against HCV. Protocols for the manuf. and
resolubilization of the enzyme as inclusion bodies in Escherichia coli are
described in detail.

=> d17 6 bib abs

L7 ANSWER 6 OF 8 CA COPYRIGHT 2003 ACS
AN 111:152144 CA

TI Recovery of proteins from cells using surfactants

IN Patroni, Joseph John; Brandon, Malcolm Roy

PA Bunge (Australia) Pty. Ltd., Australia

SO Eur. Pat. Appl., 5 pp.

CODEN: EPXXDW

DT Patent

LA English

FAN CNT 1

PATENT NO. KIND DATE APPLICATION NO. DATE

PI EP 295859 A2 19881221 EP 1988-305404 19880614

EP 295859 A3 19900418

EP 295859 B1 19941117

R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE

AU 8817632 A1 19881215 AU 1988-17632 19880610

AU 609824 B2 19910509

ZA 8804212 A 19890329 ZA 1988-4212 19880613

US 4992531 A 19910212 US 1988-206006 19880613

DK 8803221 A 19881217 DK 1988-3221 19880614

CA 1305284 A1 19920714 CA 1988-569448 19880614

ES 2065911 T3 19950301 ES 1988-305404 19880614

NO 8802635 A 19881219 NO 1988-2635 19880615

CN 88103725 A 19881228 CN 1988-103725 19880615

CN 1032425 B 19960731

JP 01047389 A2 19890221 JP 1988-147909 19880615

KR 126767 B1 19971229 KR 1988-7253 19880616

PRAI AU 1987-2472 A 19870615

AB Proteins in host cells, e.g. those in ***inclusion*** bodies, are
recovered using cationic, anionic, or zwitterionic surfactants.
Escherichia coli contg. methionine-porcine growth hormone in
inclusion bodies were treated with 20 wt./vol. %
cetyltrimethylammonium bromide for 1 h at room temp. Upon centrifugation,
a pellet was obtained which contained no ***inclusion*** bodies (as

detd. by electron microscopy).

=> file reg
 COST IN U.S. DOLLARS SINCE FILE TOTAL
 FULL ESTIMATED COST ENTRY SESSION 20.13 24.96
 DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS) SINCE FILE
 TOTAL

CA SUBSCRIBER PRICE ENTRY SESSION
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 FILE 'REGISTRY' ENTERED AT 14:30:58 ON 04 MAR 2003
 => d 11

L1 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2003 ACS
 RN 1643-20-5 REGISTRY
 CN 1-Dodecanamine, N,N-dimethyl-, N-oxide (9CI) (CA INDEX NAME)
 OTHER CA INDEX NAMES:
 CN Dodecylamine, N,N-dimethyl-, N-oxide (6CI, 8CI)
 OTHER NAMES:
 CN 101CG
 CN Admox 12
 CN Annonyx AO
 CN Annonyx C10 Amine Oxide
 CN Annonyx DMCD 40
 CN Annonyx LO
 CN Amphitol 20N
 CN Aromox DM 12D
 CN Aromox DM 12D-W
 CN Aromox DM 12W
 CN Aromox DMCD
 CN Atlas CD 413
 CN Conco XAL
 CN Cyclomox L
 CN DDNO
 CN Dimethyl/laurylamine oxide
 CN Dimethyl/dodecylamine oxide
 CN Dimethyl/laurylamine oxide
 CN Dodecyl/dimethylamine oxide
 CN Emal 20N
 CN Emcol L
 CN Emcol LO
 CN Empigen OB
 CN Ictronine oxide L
 CN Lauramine oxide
 CN Laurylamine oxide
 CN Lauryl/dimethylamine N-oxide
 CN Lauryl/dimethylamine oxide

CN ***LDAO***
 CN N,N-Dimethyl-1-dodecanamine oxide
 CN N,N-Dimethyl-n-dodecylamine oxide
 CN N,N-Dimethyl/dodecylamine oxide
 CN N-Dodecyl-N,N-dimethylamine oxide
 CN n-Dodecyl/dimethylamine oxide
 CN N-Lauryl-N,N-dimethylamine oxide
 CN N-Lauryl/dimethylamine N-oxide
 CN Ninox DMCD 40
 CN Ninox L
 CN Nissan Unisafe A-LM
 CN Oxamin LO
 CN Oxidel DM 20
 CN Rewominox L 408
 CN Rhodamox L
 CN Rhodamox LO
 CN Schercarnox DML
 CN Softamine L
 CN Tomah AO 728
 CN Unisafe A-LM
 FS 3D CONCORD
 DR 177162-47-9, 163221-07-6, 135526-66-8, 73502-08-6, 160714-02-3,
 311814-25-2
 MF C14 H31 N O
 CI COM
 LC STN Files: AGRICOLA, ANABSTR, BELLSTEIN*, BIOBUSINESS, BIOSIS,
 BIOTECHNO, CA, CANCERLIT, CAOLD, CAPLUS, CASREACT, CHEMCATS,
 CHEMLIST,
 CSCEM, DETHERM*, EMBASE, HSDB*, IFICDB, IFIPAT, IFIUDB, IPA,
 MEDLINE,
 MSDS-OHS, NIOSHTIC, PROMT, RTECS*, SPECINFO, TOXCENTER,
 USPAT2,
 USPATFUL

(*File contains numerically searchable property data)
 Other Sources: DSL**, EINECS**, TSCA**
 (**Enter CHEMLIST File for up-to-date regulatory information)

/ Structure 1 in file .gra /
 1571 REFERENCES IN FILE CA (1962 TO DATE)
 12 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
 1574 REFERENCES IN FILE CAPLUS (1962 TO DATE)
 21 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

=> log hold
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 FULL ESTIMATED COST 2.08 27.04

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS) SINCE FILE
TOTAL

ENTRY SESSION

CA SUBSCRIBER PRICE 0.00 -1.24

SESSION WILL BE HELD FOR 60 MINUTES

STN INTERNATIONAL SESSION SUSPENDED AT 14:31:33 ON 04 MAR 2003

***** RECONNECTED TO STN INTERNATIONAL *****

SESSION RESUMED IN FILE 'REGISTRY' AT 14:33:36 ON 04 MAR 2003

FILE 'REGISTRY' ENTERED AT 14:33:36 ON 04 MAR 2003

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COST IN U.S. DOLLARS SINCE FILE TOTAL

ENTRY SESSION

FULL ESTIMATED COST 2.08 27.04

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS) SINCE FILE
TOTAL

ENTRY SESSION

CA SUBSCRIBER PRICE 0.00 -1.24

FILE 'REGISTRY' ENTERED AT 14:30:58 ON 04 MAR 2003

=> FIL REGISTRY

COST IN U.S. DOLLARS SINCE FILE TOTAL

ENTRY SESSION

FULL ESTIMATED COST 3.76 28.72

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS) SINCE FILE
TOTAL

ENTRY SESSION

CA SUBSCRIBER PRICE 0.00 -1.24

FILE 'REGISTRY' ENTERED AT 14:33:46 ON 04 MAR 2003

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STRUCTURE FILE UPDATES: 3 MAR 2003 HIGHEST RN 496834-05-0

DICTIONARY FILE UPDATES: 3 MAR 2003 HIGHEST RN 496834-05-0

TSCA INFORMATION NOW CURRENT THROUGH MAY 20, 2002

=> SET TERMSET E#

SET COMMAND COMPLETED

=> DEL SEL Y

=> SEL L1 I RN

E1 THROUGH E1 ASSIGNED

=> S E1/RN

L8 1 1643-20-5/RN

=> SET TERMSET LOGIN

SET COMMAND COMPLETED

=> FIL MEDLINE

COST IN U.S. DOLLARS SINCE FILE TOTAL

ENTRY SESSION

FULL ESTIMATED COST 0.48 29.20

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS) SINCE FILE
TOTAL

ENTRY SESSION

CA SUBSCRIBER PRICE 0.00 -1.24

FILE 'MEDLINE' ENTERED AT 14:33:51 ON 04 MAR 2003

FILE LAST UPDATED: 2 MAR 2003 (20030302/UP). FILE COVERS 1958 TO
DATE.

On June 9, 2002, MEDLINE was reloaded. See HELP RLOAD for details.

MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the

MeSH 2003 vocabulary. See <http://www.nlm.nih.gov/mesh/sumn2003.html>

for a description on changes.

This file contains CAS Registry Numbers for easy and accurate
substance identification.

=> SL8

L9 59 L8

=> s purif? and l9

609884 PURIF?

L10 20 PURIF? AND L9

=> d L10 b abs

L10 ANSWER 2 OF 20 MEDLINE

AN 2002209524 MEDLINE

DN 21940604 PubMed ID: 11943219

TI Self-assembly of ATP synthase subunit c rings.

AU Arechaga Ignacio, Butler P Jonathan G, Walker John E

CS The Medical Research Council Dunn Human Nutrition Unit, Hills Road, CB2

2YK, Cambridge, UK.

SO FEBS LETTERS, (2002 Mar 27) 515 (1-3) 189-93.

Journal code: 0155157. ISSN: 0014-5793.

CY Netherlands

DT Journal, Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 200205

ED Entered STN: 20020412

Last Updated on STN: 20020511

Entered Medline: 20020510

AB Subunit c of the H(+) transporting ATP synthase is an essential part of
its membrane domain that participates in transmembrane proton conduction.

The annular architecture of the subunit c from different species has been

previously reported. However, little is known about the type of

interactions that affect the formation of c-rings in the ATPase complex.

Here we report that subunit c over-expressed in *Escherichia coli* and

purified in non-ionic detergent solutions self-assembles into annular structures in the absence of other subunits of the complex. The results suggest that the ability of subunit c to form rings is determined by its primary structure.

=> log hold

COST IN U.S. DOLLARS	ENTRY	SESSION	SINCE FILE	TOTAL
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TOTAL	ENTRY	SESSION		
CA SUBSCRIBER PRICE		0.00	-1.24	
SESSION WILL BE HELD FOR 60 MINUTES				
STN INTERNATIONAL SESSION SUSPENDED AT 14:36:01 ON 04 MAR 2003				

b 155

04mar03 14:56:02 User208669 Session D2221.1
 \$0.36 0.103 DialUnits File1
 \$0.36 Estimated cost File1
 \$0.36 Estimated cost this search
 \$0.36 Estimated total session cost 0.103 DialUnits

File 155:MEDLINE(R) 1966-2003/Feb W4
 (c) format only 2003 The Dialog Corp.

Set Items Description

?ds
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 Set Items Description
 S1 25199 HCV OR HEPATITIS(W)C
 S2 0 NS2/3(W)PROTEASE
 S3 15 NS2(2W)PROTEASE
 S4 50 NS2(W)3
 S5 73251 PROTEASE OR PROTEINASE
 S6 25 S4 AND S5
 ?t s6/7/3 5 6 13 18-20
 6/7/3

DIALOG(R)File 155:MEDLINE(R)
 (c) format only 2003 The Dialog Corp. All rts. reserv.
 12710074 21588263 PMID: 11591719

In vitro characterization of a purified NS2/3 protease variant of hepatitis C virus.

Thibeault D; Maurice R; Pilote L; Lamarre D; Pause A
 Department of Biological Sciences, Boehringer Ingelheim (Canada) Ltd.,
 Research and Development, Laval, Quebec H7S 2G5, Canada.
 dthibeault@lav.boehringer-ingelheim.com
 Journal of biological chemistry (United States) Dec 7 2001, 276 (49)
 p46678-84, ISSN 0021-9258 Journal Code: 2985121R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The cleavage of the hepatitis C virus polyprotein between the nonstructural proteins NS2 and NS3 is mediated by the NS2/3 protease, whereas the NS3 protease is responsible for the cleavage of the downstream proteins. Purification and in vitro characterization of the NS2/3 protease has been hampered by its hydrophobic nature. NS2/3 protease activity could only be detected in cells or in in vitro translation assays with the addition of microsomal membranes or detergent. To facilitate purification of this poorly characterized protease, we truncated the N-terminal hydrophobic domain, resulting in an active enzyme with improved biophysical properties. We define a minimal catalytic region of NS2/3 protease

retaining autocleavage activity that spans residues 904-1206 and includes the C-terminal half of NS2 and the N-terminal NS3 protease domain. The NS2/3 (904-1206) variant was purified from *Escherichia coli* inclusion bodies and refolded by gel filtration chromatography. The purified inactive form of NS2/3 (904-1206) was activated by the addition of glycerol and detergent to induce autocleavage at the predicted site between Leu(1026) and Ala(1027). NS2/3 (904-1206) activity was dependent on zinc ions and could be inhibited by NS4A peptides, peptides that span the cleavage site, or an N-terminal peptidic cleavage product. This NS2/3 variant will facilitate the development of an assay suitable for identifying inhibitors of HCV replication.

Record Date Created: 20011203

6/7/5

DIALOG(R)File 155:MEDLINE(R)

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11362000 21443988 PMID: 11559826

Characterization of the hepatitis C virus NS2/3 processing reaction by using a purified precursor protein.

Pallaoro M; Lahm A; Biasiol G; Brunetti M; Nardella C; Orsatti L; Bonelli F; Orru S; Nargès F; Steinkuhler C

Department of Biochemistry, Istituto di Ricerche di Biologia Molecolare "P. Angeletti," Pomezia, Italy.

Journal of virology (United States) Oct 2001, 75 (20) p9939-46,

ISSN 0022-538X Journal Code: 0113724

Document type: Journal Article

Language: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The NS2-NS3 region of the hepatitis C virus polyprotein encodes a proteolytic activity that is required for processing of the NS2/3 junction.

Membrane association of NS2 and the autocatalytic nature of the NS2/3 processing event have so far constituted hurdles to the detailed investigation of this reaction. We now report the first biochemical characterization of the self-processing activity of a purified NS2/3 precursor. Using multiple sequence alignments, we were able to define a minimal domain, devoid of membrane-anchoring sequences, which was still capable of performing the processing reaction. This truncated protein was efficiently expressed and processed in *Escherichia coli*. The processing reaction could be significantly suppressed by growth in minimal medium in the absence of added zinc ions, leading to the accumulation of an unprocessed precursor protein in inclusion bodies. This protein was purified to homogeneity, refolded, and shown to undergo processing at the authentic NS2/NS3 cleavage site with rates comparable to those observed using an in vitro-translated full-length NS2/3 precursor. Size-exclusion chromatography and a dependence of the processing rate on the concentration of truncated NS2/3 suggested a functional multimerization of the precursor

protein. However, we were unable to observe trans cleavage activity between cleavage-site mutants and active-site mutants. Furthermore, the cleavage reaction of the wild-type protein was not inhibited by addition of a mutant that was unable to undergo self-processing. Site-directed mutagenesis data and the independence of the processing rate from the nature of the added metal ion argue in favor of NS2/3 being a cysteine protease having Cys993 and His952 as a catalytic dyad. We conclude that a purified protein can efficiently reproduce processing at the NS2/3 site in the absence of additional cofactors.

Record Date Created: 20010917

6/7/6

DIALOG(R)File 155:MEDLINE(R)

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11339775 21405473 PMID: 11513846

Reconstitution of hepatitis C virus protease activities in yeast.

Mak P; Palant O; Labonte P; Plotch S

Molecular Biology and Virology Section, Wyeth-Ayerst Research, 401 N. Middletown Road, Pearl River, NY 10965, USA. makp@war.wyeth.com
FEBS letters (Netherlands) Aug 10 2001, 503 (1) p13-8, ISSN 0014-5793 Journal Code: 0155157

Document type: Journal Article

Language: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The hepatitis C virus (HCV) protease genes (NS2/3 and NS3) were expressed in yeast with their natural substrates fused to a ligand-dependent transcriptional activator, the retinoic acid receptor (RARbeta). RARbeta can activate transcription in yeast cells in response to retinoic acids. We hypothesized that cis-cleavage at the NS2-3 or NS3-4A junctions by the appropriate HCV proteases would release RARbeta, thereby activating transcription of a reporter gene. Our results from Western blot analyses and reporter gene activation indicate that the wild-type NS2/3 and NS3 enzymes are catalytically active in yeast cells, whereas mutations in the catalytic domain of NS2(C993V) and NS3(S1165A) lead to inactive enzymes. We conclude that HCV NS2/3 and NS3 protease activities can be reconstituted in yeast.

Record Date Created: 20010821

6/7/13

DIALOG(R)File 155:MEDLINE(R)

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10142656 99127866 PMID: 9930676

Engineering, characterization and phage display of hepatitis C virus NS3 protease and NS4A cofactor peptide as a single-chain protein.

Dinasi N; Pasquo A; Martin F; Di Marco S; Steinkuhler C; Cortese R; Sollazzo M

Istituto di Ricerche di Biologia Molecolare, Pomezia (Rome), Italy.
 Protein engineering (ENGLAND) Dec 1998, 11 (12) p1257-65, ISSN
 0269-2139 Journal Code: 8801484

Document type: Journal Article

Language: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The polyprotein encoded by hepatitis C virus (HCV) genomic RNA is processed into functional polypeptides by both host- and virus-encoded proteases. The HCV-encoded NS3 protease and its cofactor peptide NS4A form a non-covalent complex, which participates in processing the viral polyprotein. This proteolytic activity is believed to be essential for virus proliferation and thus the NS3 protease is a prime target for developing anti-HCV pharmacological agents. Recent X-ray crystallography structural studies have revealed the nature of this non-covalent complex between NS3 protease and the 'active' central segment of NS4A, providing the opportunity to design a single-chain polypeptide. To this end, the DNA sequence encoding for the NS4A peptide (residues 21-34) was genetically fused via a short linker, capable of making a beta-turn, to the N-terminus of the NS3 protease domain. This engineered single-chain NS3-protease (scNS3) is fully active with kinetic parameters virtually identical with those of the NS3/NS4A non-covalent complex. Moreover, the scNS3 protease can be displayed on filamentous phage and affinity selected using an immobilized specific inhibitor. The scNS3 expressed as a soluble protein and in a phage-display format facilitates enzyme engineering for further structural studies and in vitro selection of potential drug-resistant mutants. These are important steps towards developing effective anti-protease compounds.

Record Date Created: 19990323

6/7/18

DIALOG(R)File 155:MEDLINE(R)

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09698910 98111692 PMID: 9450039

Hepatitis C virus NS2-3 proteinase.

Wilkinson C S

Roche Discovery Welwyn, Welwyn Garden City, Herts, England.

Biochemical Society transactions (ENGLAND) Nov 1997, 25 (4) pS611,
 ISSN 0300-5127 Journal Code: 7506897

Document type: Journal Article

Language: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Record Date Created: 19980413

6/7/19

DIALOG(R)File 155:MEDLINE(R)

(c) format only 2003 The Dialog Corp. All rts. reserv.
 09504675 97404642 PMID: 9261354

In vitro study of the NS2-3 protease of hepatitis C virus.

Pieroni L, Santolini E, Fipaldini C, Pacini L, Migliaccio G, La Monica N
 I.R.B.M. Istituto di Ricerche di Biologia Molecolare P. Angeletti,
 Italy.

Journal of virology (UNITED STATES) Sep 1997, 71 (9) p6373-80,
 ISSN 0022-538X Journal Code: 0113724

Document type: Journal Article

Language: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Processing at the C terminus of the NS2 protein of hepatitis C virus (HCV) is mediated by a virus-encoded protease which spans most of the NS2 protein and part of the NS3 polypeptide. In vitro cotranslational cleavage at the 2-3 junction is stimulated by the presence of microsomal membranes and ultimately results in the membrane insertion of the NS2 polypeptide. To characterize the biochemical properties of this viral protease, we have established an in vitro assay whereby the NS2-3 protease of HCV BK can be activated posttranslationally by the addition of detergents. The cleavage proficiency of several deletion and single point mutants was the same as that observed with microsomal membranes, indicating that the overall sequence requirements for proper cleavage at this site are maintained even under these artificial conditions. The processing efficiency of the NS2-3 protease varied according to the type of detergent used and its concentration. Also, the incubation temperature affected the cleavage at the 2-3 junction. The autoproteolytic activity of the NS2-3 protease could be inhibited by alkylating agents such as iodoacetamide and N-ethylmaleimide. Metal chelators such as EDTA and phenanthroline also inhibited the viral enzyme. The EDTA inhibition of NS2-3 cleavage could be reversed, at least in part, by the addition of ZnCl₂ and CdCl₂. Among the common protease inhibitors tested, tosyl phenylalanyl chloromethyl ketone and soybean trypsin inhibitor inactivated the NS2-3 protease. By means of gel filtration analysis, it was observed that the redox state of the reaction mixture greatly influenced the processing efficiency at the 2-3 site and that factors present in the rabbit reticulocyte lysate, wheat germ extract, and HeLa cell extract were required for efficient processing at this site. Thus, the in vitro assay should allow further characterization of the biochemical properties of the NS2-3 protease of HCV and the identification of host components that contribute to the efficient processing at the 2-3 junction.

Record Date Created: 19970917

6/7/20

DIALOG(R)File 155:MEDLINE(R)

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09281493 97201561 PMID: 9049347

Identification of the protease domain in NS3 of hepatitis C virus.

Han D S, Hahn B, Rho H M, Jang S K

Department of Life Science, Pohang University of Science and Technology,
Kyungbuk, Korea.

Journal of general virology (ENGLAND) Apr 1995, 76 (Pt 4) p985-93,
ISSN 0022-1317 Journal Code: 0077340

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

NS3 of hepatitis C virus (HCV) is a serine protease that carries out the proteolytic processing of the nonstructural proteins of the HCV polyprotein. Deletion analysis of the N terminus of NS2,3,4 fusion protein revealed that the N-terminal boundary of the active protease resides between amino acids 1050 and 1083. The processing patterns of internal deletion mutants of NS2,3,4 indicated that the C terminus of the enzymically active protease resides between amino acids 1115 and 1218. The N- and C-terminal boundaries of the protease were also confirmed by determining the trans-cleavage activity of internally deleted NS3,4. NS3 protease activity was inhibited by Cu²⁺ but was slightly enhanced by Zn²⁺. This report provides a possible approach for development of antiviral agents based on protease inhibitors.

Record Date Created: 19970331

? log hold

04mar03 15:03:28 User208669 Session D2221.2

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\$1.86 TELNET

\$6.90 Estimated cost this search

\$7.26 Estimated total session cost 1.217 DialUnits

Logoff: level 02.12.60 D 15:03:28